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Blood cell sorting including sampling and cDNA libraries

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An abbreviated version of this protocol was published in Science in Dec 2019

A genome-wide transcriptomic analysis of protein-coding genes in human blood cells

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Detailed protocol

Dear Özgür,

Please, find the list of the antibodies bellow together with a gating strategy attached as PDF.

List of antibodies used for cell sorting:

CD194/CCR4 [L291H4] PE (Biolegend, cat#359411); CD11c [Bu15] Brilliant Violet 421 (Biolegend, cat#337225); CD123 [6H6] FITC (Biolegend, cat#306014); CD127 [A019D5] Brilliant Violet 785 (Biolegend, cat#351329); CD14 [HCD14] PE (Biolegend, cat#325606); CD14 [HCD14] FITC (Biolegend, cat#325603); CD15 [MMA] eFluor 450 (eBioscience, cat#48- 0158-41); CD16 [3G8] Brilliant Violet 711 (Biolegend, cat#302044); CD161 [HP-3G10] Brilliant Violet 421 (Biolegend, cat#339913); CD19 [HIB19] PE/Cy7 (Biolegend, cat#302216); CD193/CCR3 [5E8-G9-B4] APC (eBioscience, cat#17-1939-42); CD20 [2H7] Brilliant Violet 650 (Biolegend, cat#302336); CD203c [NP4D6] PE (eBioscience, cat#12-2039-41); CD25 [M- A251] Brilliant Violet 421 (Biolegend, cat#356113); CD27 [L128] Brilliant Violet 650 (BD biosciences, cat#563228); CD3 [HIT3a] Alexa Fluor 700 (Biolegend, cat#300324); CD3 [UCHT1] BD Horizon V450 (BD biosciences, cat#560366); CD3 [UCHT1] APC/Fire 750 (Biolegend, cat#300469); CD38 [HB-7] FITC (Biolegend, cat#356609); CD38 [HIT2] PE/Cy5 (eBioscience, cat#15-0389-42); CD4 [RPA-T4] PE/Cy5 (Biolegend, cat#300510); CD45 [HI30] BUV395 (BD biosciences, cat#563792); CD45RA [HI100] Brilliant Violet 785 (Biolegend, cat#304140); CD56 [5.1H11] APC (Biolegend, cat#362503); CD56 [NCAM16.2] Brilliant Violet 421 (BD biosciences, cat#562752); CD8a [SK1] Alexa Fluor 488 (Biolegend, cat#344716); HLA-Dr [L243] Brilliant Violet 605 (Biolegend, cat#307640); TCR V□7.2 [3C10] PE (Biolegend, cat#351705); □δTCR [B1.1] PerCP-eFluor 710 (eBioscience, cat#46-9959-41);

Panels:

DC panel: CD123[6H6] FITC; CD3[HIT3a] AF700; CD56[5.1H11] APC; CD14[HCD14] PE; CD19[HIB19] PE-Cy7; CD20[2H7] BV650; CD16[3G8] BV711; HLA-Dr[L243] BV605; CD11c[Bu15] BV421

Neutrophils/Eosinophils panel: CD38[HB-7] FITC; CD193[5E8-G9-B4] APC; CD45[HI30] BUV395; CD203c[NP4D6] PE; CD15[MMA]eF450; HLA-Dr[L243] BV605; CD16[3G8] BV711

Monocytes panel: CD3[HIT3a] AF700; CD56[5.1H11] APC; CD14[HCD14] PE; CD19[HIB19] PE-Cy7; CD20[2H7] BV650; CD16[3G8] BV711

T-cell panel: CD3[HIT3a] AF700; CD4[RPA-T4] PE-Cy5; CD8a[RPA-T8] AF488; CD45RA[HI100] BV785

Tregs panel: CD3[HIT3a] AF700; CD4[RPA-T4] FITC; CCR4[L291H4] PE; CD25[M-A251] BV421; CD127[A019D5] BV785

MAIT and gdTCR cells panel: CD19[HIB19] PE-Cy7; CD3[UCHT1] APC-Fire750; CD14[HCD14] FITC; gdTCR[B1.1] PerCp-eF710; TCR Va7.2[3C10] PE; CD161[HP-3G10] BV421

B-cell and NK cell panel: CD19[HIB19] PE-Cy7; CD3[HIT3a] AF700; CD14[HCD14] PE; CD56[NCAM16.2] BV421; CD27[L128] BV650

Basophils

The basophils were isolated employing three different approaches.

1. Isolation of the basophils from PBMCs; staining panel#1: Debris and cell aggregates were eliminated by gating based on scatter profile. The NK, T- and B-cells were eliminated by selecting CD3^{neg}/CD19^{neg}/CD20^{neg}/CD56^{neg} population. The basophils were sorted as CD123^{+}/HLA-Dr^{neg}/CD11c^{neg}/SSC^{low} sub-population. Between approx. 5,000 – 20,000 cells were sorted per sample for transcriptomic analysis by mRNA-sequencing.
2. Isolation of the basophils from full blood; staining panel#2: Erythrocytes were eliminated by HetaSep sedimentation method. Remaining erythrocytes, debris and cell aggregates were eliminated by gating on CD45^{+} events and based on scatter profile. The basophils were sorted as CD193^{+}/CD203c^{+}/CD38^{+}/CD15^{neg}/CD16^{neg}/HLA-Dr^{neg}/SSC^{low} sub-population. Between approx. 1,500 – 5,700 cells were sorted per sample for transcriptomic analysis by mRNA-sequencing.
3. Isolation of the basophils from full blood; staining panel#3: Erythrocytes were eliminated by HetaSep sedimentation method. Remaining erythrocytes, debris and cell aggregates were eliminated by gating on CD45^{+} events and based on scatter profile. The basophils were sorted as CD123^{+}/CD193^{+}/CD38^{+}/CD3^{neg}/CD11c^{neg}/ CD15^{neg}/CD16^{neg}/CD20^{neg}/CD56^{neg}/HLA-Dr^{neg}/SSC^{low}sub-population. Between approx. 2,000 – 5,800 cells were sorted per sample for transcriptomic analysis by mRNA-sequencing.

Panel#1: CD123[6H6] FITC; CD3[HIT3a] AF700; CD56[5.1H11] APC; CD14[HCD14] PE; CD19[HIB19] PE-Cy7; CD20[2H7] BV650; CD16[3G8] BV711; CD11c[Bu15] BV421
Panel#2: CD38[HB-7] FITC; CD193[5E8-G9-B4] APC; CD45[HI30] BUV395; CD203c[NP4D6] PE; CD15[MMA] eF450; HLA-Dr[L243] BV605; CD16[3G8] BV711
Panel#3: CD123[6H6] FITC; CD193[5E8-G9-B4] APC; CD45[HI30] BUV395; CD203c[NP4D6] PE; CD38[HIT2] PE-Cy5; CD15[MMA] eF450; CD3[UCHT1] BD Horizon V450; CD56[NCAM16.2] BV421; CD11c[Bu15] BV421; HLA-Dr[L243] BV605; CD20[2H7] BV650; CD16[3G8] BV711

Kind regards,
Jaromir

Related files

aax9198_Uhlen_SM_Gating scheme for cell sorting.pdf

How to cite:（Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used）

1. Uhlen, M. , Fagerberg, L. , Brodin, P. and Mikes, J. (2021). Blood cell sorting including sampling and cDNA libraries. Bio-protocol Preprint. bio-protocol.org/prep1472.
2. Uhlen, M., Karlsson, M. J., Zhong, W., Tebani, A., Pou, C., Mikes, J., Lakshmikanth, T., Forsström, B., Edfors, F., Odeberg, J., Mardinoglu, A., Zhang, C., von Feilitzen, K., Mulder, J., Sjöstedt, E., Hober, A., Oksvold, P., Zwahlen, M., Ponten, F., Lindskog, C., Sivertsson, Å., Fagerberg, L., Brodin, P. and Feilitzen, K. V.(2019). A genome-wide transcriptomic analysis of protein-coding genes in human blood cells . Science 366(6472). DOI: 10.1126/science.aax9198

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